IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Paul L. Kaufman *et al.*
Serial No.: Group No.:
Filed: Examiner:
Entitled: **Cytoskeletal Active Agents For Glaucoma Therapy**

#37
9-14-98

DECLARATION OF DR. THOMAS MITTAG

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.10

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the U.S. Postal Service on this date **February 11, 1998** in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number **EM 121 691 975 US** addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Dated: Feb. 11, 1998 By: Marlene E. Garitano
Marlene E. Garitano

Sir or Madam:

1. I, Dr. Thomas Mittag am familiar with U.S. Patent Appln. Ser. No. 08/604,568, to Kaufman and Geiger.

2. I am familiar with the Final Office Action from the Patent Office mailed May 28, 1997, in regard to U.S. Patent Appln. Ser. No. 08/604,568.

3. I am familiar with the Office Action from the Patent Office mailed November 12, 1996, in regard to U.S. Patent Appln. Ser. No. 08/604,568. I am also familiar with the Amendment and Response to the Office Action, submitted by the attorney for Drs. Kaufman and Geiger on January 30, 1997.

4. In both Office Actions, the Examiner rejected Claims 1-13 of U.S. Patent Appln. Ser. No. 08/604,568, as being obvious under two publications, namely an abstract by Nagata *et al.* ("Possible Mechanisms of Inositol Phosphate-Diacylglycerol Signalling Pathway in the Regulation of Intraocular Pressure," Acta. Soc. Ophthalmol. Jpn., 96(7):865-871

[1991]), and a scientific paper by Yoshimura *et al.* ("Analysis of Protein Kinase Activities in Rabbit Ciliary Substrates," Exp. Eye Res., 45(1):45-56 [1987]).

5. I am the Dr. Mittag listed as a co-author of the Yoshimura *et al.* paper. Indeed, the work reported in this paper was conceived and conducted in my laboratory under my supervision. Dr. Yoshimura was a visiting fellow from Kyoto University, who worked in my laboratory on the project reported in our paper. Although Dr. Yoshimura and Dr. Podos provided substantial contributions to our paper, I wrote most of our paper, and supervised its production.

6. The paper cited by the Examiner is part of a series of publications by my laboratory, which report our efforts to understand the mechanism(s) involved in regulating the **formation** of aqueous humor by the ciliary processes of the eye. Specifically, this paper characterizes one of the biochemical steps that connects receptor signals to the fluid transport systems of the ciliary process tissue, namely kinase enzyme activity.

7. We did not study, nor did we report on the tissue responsible for the *outflow* of aqueous humor, namely the trabecular tissue, nor did we characterize the kinase activities in trabecular tissue.

8. Our use of the compound H-7 was entirely for the purpose of differentiating various kinase activities in ciliary process tissue on a biochemical basis (*i.e.,* the relative degree of inhibition of various kinase activities by H-7). We did not conduct experiments with H-7 to determine cytoskeletal, cell junction/adhesion, or cell shape responses of eye tissue to this agent, nor did we do any experiments to determine the effects of H-7 on aqueous humor dynamics in the intact eye.

9. As known in the field of ophthalmology, there are anatomic and physiologic distinctions between the ciliary epithelium and the trabecular meshwork. The ciliary epithelium governs aqueous humor inflow (*i.e.,* **formation**) by active secretion, while the trabecular meshwork governs aqueous humor *outflow* by passive bulk fluid drainage.

10. It is my opinion that the Examiner has misinterpreted the work described in our paper as our publication does not "teach the effect of the claimed [compound] on the enablement of aqueous humor outflow to regulate intra-ocular pressure."

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: December 30, 1997

Signed: Thomas Mittag

Dr. Thomas Mittag

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Paul L. Kaufman *et al.*
Serial No.: Group No.:
Filed: Examiner:
Entitled: **Cytoskeletal Active Agents For Glaucoma Therapy**

DECLARATION OF DR. PAUL KAUFMAN

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.10

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the U.S. Postal Service on this date **February 11, 1998** in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number **EM 121 691 975 US** addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Dated: Feb. 11, 1998 By: Marlene E. Garitano
Marlene E. Garitano

Sir or Madam:

1. I, Dr. Paul Kaufman am a co-inventor of U.S. Patent Appln. Ser. No. 08/604,568, and the present continuation application filed herewith.

2. I am familiar with the Final Office Action from the Patent Office mailed May 28, 1997 in regard to U.S. Patent Appln. Ser. No. 08/604,568.

3. I am familiar with the Office Action from the Patent Office mailed November 12, 1996, in regard to U.S. Patent Appln. Ser. No. 08/604,568. I am also familiar with the Amendment and Response to the Office Action, submitted by our attorney on January 30, 1997.

4. As known in the field of ophthalmology, there are anatomic and physiologic distinctions between the ciliary epithelium and the trabecular meshwork. The ciliary epithelium governs aqueous humor inflow (*i.e.,* **formation**) by active secretion, while the trabecular meshwork governs aqueous humor *outflow* by passive bulk fluid drainage.

5. In addition, ion transport is involved in the **formation** of aqueous humor by a geometrically unaltered secretory epithelium (*i.e.,* ciliary epithelium), while cytoskeletal attenuation, cell junction weakening, and cell separation result in the passive drainage of bulk fluid through a geometrically altered resistive pathway (*i.e.,* trabecular meshwork).

6. In both Office Actions, the Examiner rejected Claims 1-13 of U.S. Patent Appln. Ser. No. 08/604,568, as allegedly failing to provide an enabling disclosure of our invention. In specific, the Examiner argues that our application fails to enable "a person skilled in the art" to use all serine-threonine kinase inhibitors and obtain the enablement of aqueous humor outflow." (Office Action mailed November, 12, 1996, p. 2). The Examiner argues that the phrase "serine threonine inhibitor" is broad, and a "person skilled in the art will not be able to arrive at the list of the compounds encompassed under such phrase." (Final Office Action, dated May 27, 1997, p. 2).

7. The present application provides methods for the use of certain serine threonine kinase inhibitors on aqueous humor outflow. As indicated in the application, our claims are directed to members of the serine threonine kinase inhibitor class of compounds that are effective at enhancing aqueous humor outflow in the eye (*See e.g.,* page 24, lines 14-16). Furthermore, we provide screening methods that are suitable for identifying serine threonine kinase inhibitors with this effect (*See,* page 25, lines 11-13; and the Examples). These methods for initial evaluation of serine-threonine kinases include: (1) determining the safety of the test compounds using corneal endothelial cells; (2) determining the effect of the compound on overall ocular tolerability/toxicity; (3) examining the *in vitro* effect of compounds on vascular and/or trabecular meshwork endothelium in culture, as well as live monkey intraocular pressure and outflow studies; (4) determining the effect of topically administered compound on the intraocular pressure by non-invasive measurement techniques (*e.g.*, applanation tonometry).

8. Compounds that show promise can then be examined in a more comprehensive screening procedure that includes the steps of I) Determining the effectiveness of the compound on outflow facility in living monkeys; II) Documenting whether compound is acting directly on the trabecular meshwork in living monkeys with disinserted ciliary muscle;

III) Performing electron microscopy to analyze the effect of the compounds on the structure of the trabecular meshwork and other tissues; IV) Determining the effect of the compound on other properties, including refraction, accommodative and pupillary responses to pilocarpine; and corneal endothelial cell counts and morphology; and V) Consideration of clinical trials on human subjects. In addition to these steps described in the Specification, tracer studies may also be conducted in making the determination of whether compounds act directly on the trabecular meshwork in living monkeys with or without disinserted ciliary muscle.

9. Using these screening methods, we demonstrated that effective serine threonine kinase inhibitors can be distinguished from ineffective compounds (*See e.g.,* the comparative experiments and data on ML-7, chelerythrine, and various tyrphostins presented in Tables 1-3, attached hereto at Tabs 1-3, respectively). As shown in these data, ML-7 at doses relatively specific for myosin light chain kinase inhibition significantly increased (*i.e.,* a statistically significant increase >30% relative to baseline values and adjusted for perfusion-induced control eye resistance washout) outflow facility. Neither chelerythrine (at doses that would be relatively specific for PKC inhibition) nor various tyrphostins (at doses which inhibit diverse tyrosine kinases) significantly affected outflow facility. This reflects our explicit definition of "serine-threonine kinase inhibitor" in our application (*See,* page 9, lines 1-7; and the detailed discussion of this class of compounds on page 26 line 25, through page 29, line 20).

10. Our claims focus on kinase inhibitors that effect cell adhesion and the actin cytoskeleton (*e.g.,* H-7, ML-7, and staurosporine). Our work shows that these compounds dramatically effect cell contractility and increase aqueous humor *outflow* facility via their junction and cytoskeleton-disrupting activities.

11. In both Office Actions, the Examiner rejected Claims 1-13 of U.S. Patent Appln. Ser. No. 08/604,568, as being obvious under two publications, namely an abstract by Nagata *et al.* ("Possible Mechanisms of Inositol Phosphate-Diacylglycerol Signalling Pathway in the Regulation of Intraocular Pressure," Acta. Soc. Ophthalmol. Jpn., 96(7):865-871 [1991]), and a scientific paper by Yoshimura *et al.* ("Analysis of Protein Kinase Activities in Rabbit Ciliary Substrates," Exp. Eye Res., 45(1):45-56 [1987]). I have reviewed both of these publications thoroughly.

12. The Nagata *et al.* and Yoshimura *et al.* references describe the effects of protein kinase C (PKC) and other components of signalling pathways in the ciliary epithelium putatively involved in regulation of ion transport and consequent aqueous humor **formation** or inflow, into the posterior chamber of the eye. There is no mention of enhancement of aqueous humor *outflow* from the anterior chamber via the trabecular meshwork, or of cell junctions or cytoskeletal proteins.

13. In contrast to Nagata *et al.* and Yoshimura *et al.*, our claimed invention encompasses the enhancement of aqueous humor *outflow* (*i.e.,* from the anterior chamber via the trabecular meshwork by modulation of cytoskeletal proteins and cell junctions). Our invention does not involve any effects on ion transport, aqueous humor formation, or other biological processes in the ciliary epithelium of the posterior chamber. Indeed, we have data showing that H-7 has **no effect** on aqueous humor **formation** in the live monkey, and that the H-7 effect on the cytoskeleton and cell junctions does not involve the protein kinase C pathway. Experiments and data on aqueous humor formation, showing the results of experiments in which 400 mM H-7 or vehicle was administered topically to opposite eyes in six anesthetized monkeys are attached at Tab 4 for the Examiner's review. The data on the non-relevance of the PKC pathway for the cytoskeleton and cell junctional effects of H-7 in culture were discussed in the Examples of the Specification (*See e.g.,* Examples 1-6), as well as in a publication co-authored by my co-inventor, Dr. Geiger (T. Volberg *et al.*, "Effect of protein kinase inhibitor H-7 on the contractility, integrity, and membrane anchorage of the microfilament system," Cell Motil. Cytoskel. 29:321-338 [1994][attached hereto at Tab 6]). Data on the non-relevance of the PKC for the outflow facility effect of H-7 in live monkeys (*See e.g.,* the comparative data on ML-7 and chelerythrine presented in Tables 1-2, attached hereto at Tabs 1 and 2, respectively), are discussed in paragraph 9 above.

14. As described in paragraph 13 above, Table 4 (at Tab 4) shows the results of experiments to determine the effect of topical H-7 on aqueous humor flow (AHF). Table 5 (at Tab 5), shows the results of experiments to determine the corneal endothelial transfer coefficient (k_a), representing the corneal endothelial permeability to fluorescein measured by fluorophotometry. An increased transfer coefficient represents an impairment of the endothelial barrier function. As indicated in the description of the experiment preceding the

Table, H-7 does not increase corneal endothelial permeability, and does not affect aqueous humor formation.

15. Further in contrast to Nagata *et al.*, who describe the involvement of protein kinase C (PKC) in regulating intraocular pressure, we do not suggest, nor claim any involvement of PKC in the effect of inhibitors (*e.g.*, H-7) on the actin cytoskeleton, cell adhesion, or *outflow* facility. Indeed, we have evidence that specific PKC inhibitors have no such an effect on the actin cytoskeleton, cell adhesion or *outflow* facility. These data were summarized in a poster which was presented at the Association for Research in Vision and Ophthalmology Investigative Ophthalmology and Visual Science Meeting held May 11-16, 1997. This poster includes myself and my co-inventor, Dr. Geiger, as co-authors. A copy of the abstract briefly describing this poster is attached at Tab 7. In addition, my co-inventor Dr. Geiger has published data that show that PKC inhibitors have no effect on the actin cytoskeleton in cultured non-ocular endothelial cells (*See,* Volberg *et al.*, at Tab 6).

16. Yoshimura *et al.* report studies on protein kinase activity in rabbit ciliary processes. In contrast, all of our measurements relate to the trabecular meshwork/Schlemm's canal (*i.e.*, structures involved in aqueous humor *outflow*), and not to the ciliary process (*i.e.*, structures involved in aqueous humor **formation** or inflow). Yoshimura *et al.* characterized a battery of eight different kinases (*See,* Table 4 of Yoshimura *et al.*), none of which show any relevance to cell contractility, myosin phosphorylation, or *outflow* facility.

17. It is clear the that the presently claimed invention, which is focused on aqueous humor *outflow*, is very different from the disclosure of the Yoshimura *et al.* and Nagata *et al.* references, which address aqueous humor **formation** or inflow.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: 01/15/98 Signed: Paul L. Kaufman

Dr. Paul Kaufman

EFFECT OF ML-7 AND CHELERYTHRINE ON AQUEOUS HUMOR OUTFLOW FACILITY

Total ouflow facility was measured by 2-level constant pressure perfusion in living monkeys. Following 35 minute baseline analysis, the anterior chamber was exchanged with 2 ml of 5-500 μM ML-7 or 0.1-500 μM chelerythrine solution or vehicle (opposite eyes) for 10-15 minutes. The reservoirs were then immediately filled with corresponding solutions, closed for 45 minutes, and then reopened for 45 minutes of post-drug facility measurement. The vehicle used in these experiments contained 0.01-5% DMSO in standard perfusion solution (*i.e.*, Barany's solution, a phosphate-buffered glucose, calcium, and magnesium-enriched saline). Tables 1A-D, and 2A-E show the results obtained in these experiments. The aqueous humor outflow facility data in these Tables are the means ± s.e.m. for "n" animals. "Rx" refers to post-drug facility data encompassing 45 minutes beginning 45 minutes after drug administration. "BL" refers to the baseline levels. The right-most column provides the ratios for these experiments (*e.g.*, a ratio of 1.10 in this column indicates an average 10% increase in the ML-7 treated eyes relative to the baseline in those eyes, and adjusted for the percent change from baseline in the control eyes).

At 100 μM, the selective myosin light chain kinase (MLCK) inhibitor ML-7 (IC 50: 0.3 μM), but not the selective protein kinase C (PKC) inhibitor chelerythrine (IC 50: 0.66 μM), significantly increased facility (by approximately 40%) relative to control. Both drugs produced a greater facility increase (101% with chelerythrine, and 76% with ML-7) at 500 μM, but specificity may be lost at such high doses. At lower concentrations of ML-7 (5-20 μM), no significant increase in facility was observed. Control eye facility decreased by approximately 20% in the 100 and 500 μM ML-7, and 500 μM chelerythrine tests, perhaps related to the presence of DMSO in the vehicle.

TABLE 1A.
Effect of Intracameral ML-7 on Outflow Facility (μl/min/mmHg)
(Dose--5 μM, with 0.05% DMSO)(n=5)

	ML-7	Vehicle	ML-7/Vehicle
BL	0.35 ± 0.06	0.34 ±	1.03 ± 0.09
Rx	0.40 ± 0.07	0.34 ± 0.03	1.16 ± 0.14
Rx/BL	1.13 ± 0.07	1.07 ± 0.16	1.18 ± 0.21

TABLE 1B.
Effect of Intracameral ML-7 on Outflow Facility (μl/min/mmHg)
(Dose--20 μM, with 0.2% DMSO)(n=5)

	ML-7	Vehicle	ML-7/Vehicle
BL	0.44 ± 0.09	0.53 ± 0.09	0.85 ± 0.15
Rx	0.52 ± 0.13	0.63 ± 0.15	0.84 ± 0.16
Rx/BL	1.18 ± 0.15	1.20 ± 0.16	0.99 ± 0.06

TABLE 1C.
Effect of Intracameral ML-7 on Outflow Facility (μl/min/mmHg)
(Dose--100 μM, with 0.1% DMSO; n=5) or (Dose--100 μM, with 1% DMSO; n=8)

	ML-7	Vehicle	ML-7/Vehicle
BL	0.39 ± 0.05	0.42 ± 0.06	1.06 ± 0.13
Rx	0.42 ± 0.04	0.32 ± 0.04	1.44 ± 0.18**
Rx/BL	1.09 ± 0.06	0.84 ± 0.07**	1.38 ± 0.11****

TABLE 1D.
Effect of Intracameral ML-7 on Outflow Facility (μl/min/mmHg)
(Dose--500 μM, with 5% DMSO)(n=10)

	ML-7	Vehicle	ML-7/Vehicle
BL	0.33 ± 0.05	0.39 ± 0.05	0.86 ± 0.08*
Rx	0.38 ± 0.05	0.27 ± 0.02	1.49 ± 0.21**
Rx/BL	1.29 ± 0.25	0.76 ± 0.11*	1.76 ± 0.24***

* = $p<0.1$, for ratios different from 1.0 by the 2-tailed paired t-test.
** = $p<0.05$ for ratios different from 1.0 by the 2-tailed paired t-test.
*** = $p<0.02$ for ratios different from 1.0 by the 2-tailed paired t-test.
**** = $p<0.005$ for ratios different from 1.0 by the 2-tailed paired t-test.

EFFECT OF ML-7 AND CHELERYTHRINE ON AQUEOUS HUMOR OUTFLOW FACILITY

Total ouflow facility was measured by 2-level constant pressure perfusion in living monkeys. Following 35 minute baseline analysis, the anterior chamber was exchanged with 2 ml of 5-500 μM ML-7 or 0.1-500 μM chelerythrine solution or vehicle (opposite eyes) for 10-15 minutes. The reservoirs were then immediately filled with corresponding solutions, closed for 45 minutes, and then reopened for 45 minutes of post-drug facility measurement. The vehicle used in these experiments contained 0.01-5% DMSO in standard perfusion solution (*i.e.*, Barany's solution, a phosphate-buffered glucose, calcium, and magnesium-enriched saline). Tables 1A-D, and 2A-E show the results obtained in these experiments. The aqueous humor outflow facility data in these Tables are the means ± s.e.m. for "n" animals. "Rx" refers to post-drug facility data encompassing 45 minutes beginning 45 minutes after drug administration. "BL" refers to the baseline levels. The right-most column provides the ratios for these experiments (*e.g.*, a ratio of 1.10 in this column indicates an average 10% increase in the ML-7 treated eyes relative to the baseline in those eyes, and adjusted for the percent change from baseline in the control eyes).

At 100 μM, the selective myosin light chain kinase (MLCK) inhibitor ML-7 (IC 50: 0.3 μM), but not the selective protein kinase C (PKC) inhibitor chelerythrine (IC 50: 0.66 μM), significantly increased facility (by approximately 40%) relative to control. Both drugs produced a greater facility increase (101% with chelerythrine, and 76% with ML-7) at 500 μM, but specificity may be lost at such high doses. At lower concentrations of ML-7 (5-20 μM), no significant increase in facility was observed. Control eye facility decreased by approximately 20% in the 100 and 500 μM ML-7, and 500 μM chelerythrine tests, perhaps related to the presence of DMSO in the vehicle.

TABLE 2A.
Effect of Intracameral Chelerythrine on Outflow Facility (μl/min/mmHg)
(Dose--0.1 μM, with 0.01% DMSO)(n=4)

	Chelerythrine	Vehicle	Chelerythrine/ Vehicle
BL	0.36 ± 0.05	0.40 ± 0.13	1.06 ± 0.19
Rx	0.38 ± 0.07	0.39 ± 0.11	1.08 ± 0.14
Rx/BL	1.05 ± 0.10	0.99 ± 0.06	1.06 ± 0.09

TABLE 2B.
Effect of Intracameral Chelerythrine on Outflow Facility (μl/min/mmHg)
(Dose--1.0 μM, with 0.1% DMSO)(n=4)

	Chelerythrine	Vehicle	Chelerythrine/ Vehicle
BL	0.34 ± 0.07	0.28 ± 0.05	1.23 ± 0.21
Rx	0.37 ± 0.06	0.34 ± 0.05	1.10 ± 0.16
Rx/BL	1.12 ± 0.11	1.24 ± 0.14	0.94 ± 0.17

TABLE 2C.
Effect of Intracameral Chelerythrine on Outflow Facility (μl/min/mmHg)
(Dose--10 μM, with 1% DMSO) (n=4)

	Chelerythrine	Vehicle	Chelerythrine/ Vehicle
BL	0.33 ± 0.08	0.37 ± 0.07	0.88 ± 0.12
Rx	0.32 ± 0.04	0.38 ± 0.09	0.92 ± 0.14
Rx/BL	1.08 ± 0.19	1.02 ± 0.10	1.05 ± 0.13

TABLE 2D.
Effect of Intracameral Chelerythrine on Outflow Facility (μl/min/mmHg)
(Dose--100 μM, with 3% DMSO)(n=6)

	Chelerythrine	Vehicle	Chelerythrine/ Vehicle
BL	0.36 ± 0.05	0.34 ± 0.04	1.10 ± 0.21
Rx	0.34 ± 0.04	0.34 ± 0.04	1.16 ± 0.30
Rx/BL	1.06 ± 0.22	1.05 ± 0.19	1.28 ± 0.48

TABLE 2E.
Effect of Intracameral Chelerythrine on Outflow Facility (μl/min/mmHg)
(Dose--500 μM, with 4% DMSO)(n=6)

	Chelerythrine	Vehicle	Chelerythrine/ Vehicle
BL	0.41 ± 0.03	0.42 ± 0.08	1.16 ± 0.21
Rx	0.61 ± 0.10	0.29 ± 0.02	2.19 ± 0.42**
Rx/BL	1.54 ± 0.32	0.78 ± 0.11*	2.01 ± 0.33**

* = $p<0.1$, for ratios different from 1.0 by the 2-tailed paired t-test.
** = $p<0.05$ for ratios different from 1.0 by the 2-tailed paired t-test.

EFFECT OF TYRPHOSTINS ON OUTFLOW FACILITY

In these experiments, total outflow facility was measured by 2-level constant pressure perfusion in living monkeys to determine the effect of various tyrphostins on outflow facility. The data presented here represent the results obtained with four tyrphostins (AG1406, AG1498, AG1714, and AG1478). The vehicle was 0.001-4% DMSO in a standard perfusion solution (*i.e.,* Barany's solution, a phosphate-buffered glucose, calcium, and magnesium-enriched saline). Following 35 minute baseline facility (BL) measurement, anterior chambers were exchanged with 2 ml of tyrphostin solution (2 or 3 doses for each of the four tyrphostins [*i.e.,* AG1406, AG1498, AG1714, and AG1478], as shown in Tables 3A-D) in one eye or vehicles in opposite eyes for 10-15 minutes. The reservoirs were then immediately filled with corresponding solutions, closed for 45 minutes, then reopened to take a 90 minute measurement of post-drug facility (Rx). Facility data are presented as the mean ± s.e.m. (μl/min/mmHg) for "n" animals, with each animal providing data for one eye receiving tyrphostin and one eye receiving the vehicle. The ratios are unitless. The right-most column provides the ratios for these experiments (*e.g.,* a ratio of 1.10 in this column indicates an average 10% increase in the tryphostin-treated eyes relative to the baseline in those eyes, and adjusted for the percent change from baseline in the control eyes).

Although the facilities in treated eyes in some protocols were significantly increased compared to ipsilateral baselines, the increases were not significant in comparison with perfusion-induced resistance washout in contralateral control eyes, except for a physiologically small effect of 10 μM AG1478. Therefore, these compounds were essentially ineffective at the dosages/time period studied, despite activity at these concentrations in cultured vascular endothelial cell systems.

TABLE 3A.
Effect of Tyrphostin AG1406 on Outflow Facility

	Tyrphostin AG1406			Vehicle			Tyrphostin/Vehicle		
	BL	Rx	Rx/BL	BL	Rx	Rx/BL	BL	Rx	Rx/BL
1 μM Tyrphostin 0.001% DMSO (n=8)	0.35 ± 0.06	0.54 ± 0.11	1.54 ± 0.21*	0.37 ± 0.05	0.49 ± 0.06	1.38 ± 0.12*	0.97 ± 0.11	1.13 ± 0.23	1.12 ± 0.15
10 μM Tyrphostin 0.1% DMSO (n=4)	0.37 ± 0.06	0.47 ± 0.08	1.29 ± 0.29*	0.35 ± 0.04	0.44 ± 0.11	1.24 ± 0.18	1.04 ± 0.10	1.13 ± 0.18	1.08 ± 0.09
100 μM Tyrphostin 1% DMSO (n=6)	0.27 ± 0.03	0.32 ± 0.06	1.22 ± 0.21	0.31 ± 0.05	0.37 ± 0.13	1.17 ± 0.30	0.93 ± 0.10	1.17 ± 0.25	1.24 ± 0.22

TABLE 3B.
Effect of Tyrphostin AG1498 on Outflow Facility

	Tyrphostin AG1498			Vehicle			Tyrphostin/Vehicle		
	BL	Rx	Rx/BL	BL	Rx	Rx/BL	BL	Rx	Rx/BL
10 μM Tyrphostin 0.01% DMSO (n=4)	0.37 ± 0.07	0.78 ± 0.10	2.21 ± 0.21*	0.31 ± 0.03	0.67 ± 0.14	2.11 ± 0.19**	1.22 ± 0.22	1.26 ± 0.17	1.06 ± 0.09
100 μM Tyrphostin 0.1% DMSO (n=4)	0.41 ± 0.06	0.54 ± 0.07	1.31 ± 0.05**	0.45 ± 0.12	0.58 ± 0.10	1.40 ± 0.20	1.24 ± 0.54	1.12 ± 0.54	1.03 ± 0.23

TABLE 3C.
Effect of Tyrphostin AG1714 on Outflow Facility

	Tyrphostin AG1714			Vehicle			Tyrphostin/Vehicle		
	BL	Rx	Rx/BL	BL	Rx	Rx/BL	BL	Rx	Rx/BL
10 μM Tyrphostin 0.1% DMSO (n=8)	0.29 ± 0.05	0.42 ± 0.07	1.46 ± 0.17*	0.32 ± 0.05	0.43 ± 0.07	1.35 ± 0.19	0.92 ± 0.09	1.08 ± 0.41	1.17 ± 0.14
100 μM Tyrphostin 1% DMSO (n=9)	0.33 ± 0.05	0.32 ± 0.04	1.00 ± 0.07	0.27 ± 0.04	0.26 ± 0.05	0.95 ± 0.08	1.36 ± 0.20	1.61 ± 0.35	1.10 ± 0.11

TABLE 3D.
Effect of Tyrphostin AG1478 on Outflow Facility

	Tyrphostin AG1478			Vehicle			Tyrphostin/Vehicle		
	BL	Rx	Rx/BL	BL	Rx	Rx/BL	BL	Rx	Rx/BL
1 μM Tyrphostin 0.1% DMSO (n=4)	0.39 ± 0.06	0.59 ± 0.11	1.50 ± 0.12*	0.45 ± 0.05	0.64 ± 0.11	1.42 ± 0.11*	0.87 ± 0.11	0.93 ± 0.13	1.07 ± 0.08
10 μM Tyrphostin 0.1% DMSO (n=8)	0.38 ± 0.04	0.62 ± 0.07	1.63 ± 0.10***	0.44 ± 0.04	0.61 ± 0.10	1.35 ± 0.12*	0.88 ± 0.08	1.07 ± 0.09	1.24 ± 0.08*
100 μM Tyrphostin 4% DMSO (n=4)	0.29 ± 0.09	0.18 ± 0.04	0.83 ± 0.25	0.25 ± 0.05	0.25 ± 0.05	0.99 ± 0.06	1.16 ± 0.20	0.99 ± 0.44	0.83 ± 0.27

* = $p<0.05$ for ratios different from 1.0 by the 2-tailed paired t-test
** = $p<0.01$ for ratios different from 1.0 by the 2-tailed paired t-test
*** = $p<0.001$ for ratios different from 1.0 by the 2-tailed paired t-test

EFFECT OF H-7 ON AQUEOUS HUMOR FLOW (AHF)

In these experiments, six anesthetized monkeys were used to determine the effect of H-7 on aqueous humor flow (AHF). In these experiments, 400 mM H-7 or vehicle was topically administered to opposite eyes, and the AHF was measured by fluorophotometry. The vehicle was 25%DMSO in a standard perfusion solution (*i.e.,* Barany's solution, a phosphate-buffered glucose, calcium, and magnesium-enriched saline).

The AHF data in Tables 4A-C are the means ± s.e.m. μl/min for the indicated post-treatment time intervals or their baseline counterparts. The ratios are unitless. The Baseline value is the average of baseline AHF determined one (1) week before and two (2) weeks after treatment. The "*" indicates a value of $p<0.05$, by the 2-tailed t-test for a ratio significantly different from 1.0. The right-most column provides the ratios for these experiments (*e.g.,* a ratio of 1.10 in this column indicates an average 10% increase in the H-7 treated eyes relative to the baseline in those eyes, and adjusted for the percent change from baseline in the control eyes).

When compared to ipsilateral baselines, post-drug AHF in H-7 treated eyes was increased by 43% ($p>0.05$), 19% ($p<0.05$), and 26% ($p>0.05$) respectively, during the post-treatment time intervals indicated. However, AHFs in contralateral vehicle-treated eyes were also increased by 25% ($p>0.05$), 22% ($p>0.05$), and 23% ($p>0.05$), respectively. Thus, post-drug AHF in H-7 treated eyes, relative to contralateral vehicle-treated eyes (corrected for corresponding baselines), was only increased by 23% ($p>0.05$), 5% ($p>0.05$), and 6% ($p>0.05$) respectively, during the post-treatment time intervals indicated. Thus, post-drug AHF in H-7 treated eyes, relative to contralateral vehicle-treated eyes (corrected for corresponding baselines) was not statistically significantly altered during the time indicated.

TABLE 4A.
Effect of 400 mM Topical H-7 on Aqueous Humor Flow (AHF)
At 0.5-3.0 Hours

	H-7	Vehicle	H-7/Vehicle
Baseline	1.64 ± 0.32	1.64 ± 0.20	1.01 ± 0.09
Post-Drug AHF	2.36 ± 0.60	2.13 ± 0.66	1.19 ± 0.10
Post-Drug AHF/Baseline	1.43 ± 0.23	1.25 ± 0.26	1.23 ± 0.17

TABLE 4B.
Effect of 400 mM Topical H-7 on Aqueous Humor Flow (AHF)
At 3.5-6.0 Hours

	H-7	Vehicle	H-7/ Vehicle
Baseline	1.98 ± 0.24	1.75 ± 0.15	1.12 ± 0.07
Post-Drug AHF	2.30 ± 0.20	2.21 ± 0.47	1.15 ± 0.12
Post-Drug AHF/Baseline	1.19 ± 0.06*	1.22 ± 0.15	1.05 ± 0.13

TABLE 4C.
Effect of 400 mM Topical H-7 on Aqueous Humor Flow (AHF)
At 0.5-6.0 Hours

	H-7	Vehicle	H-7/Vehicle
Baseline	1.82 ± 0.20	1.67 ± 0.16	1.09 ± 0.06
Post-Drug AHF	2.33 ± 0.42	2.16 ± 0.51	1.14 ± 0.08
Post-Drug AHF/Baseline	1.26 ± 0.11	1.23 ± 0.17	1.06 ± 0.11

EFFECT OF 400 mM TOPICAL H-7 ON CORNEAL ENDOTHELIAL TRANSFER COEFFICIENT (k_a)

As the endothelial transfer coefficient (k_a) represents the corneal endothelial permeability to fluorescein, an increased transfer coefficient represents an impairment of the endothelial barrier function. In experiments to determine the effects of H-7 on endothelial barrier function, 400 mM H-7 or vehicle were topically administered to opposite eyes, and the k_a was measured by fluorophotometry. The vehicle was 25% DMSO in a standard perfusion solution (*i.e.,* Barany's solution, a phosphate-buffered glucose, calcium, and magnesium-enriched saline). Six anesthetized monkeys were studied in these experiments.

The data in Tables 5A-C are expressed as the mean ± s.e.m. 10^{-3}/min for the indicated post-treatment time intervals or their baseline counterparts; the ratios are unitless. The right-most column provides the ratios for these experiments (*e.g.,* a ratio of 1.10 in this column indicates an average 10% increase in the H-7 treated eyes relative to the baseline in those eyes, and adjusted for the percent change from baseline in the control eyes). The baseline shown is the average of baseline k_a determined 1 week before and 2 week after treatment. As indicated by these data, compared to ipsilateral baselines, post-drug k_a in H-7-treated eyes changed by -2% ($p>0.05$), -3% ($p>0.05$) and -1% ($p>0.05$) respectively, during the post-treatment time intervals indicated. k_a in vehicle-treated eyes changed by -2% ($p>0.05$), -2% ($p>0.05$) and 2% ($p>0.05$) respectively. Post-drug k_a in H-7-treated eyes, relative to contralateral vehicle-treated eyes and corrected for corresponding baselines, changed by 5% ($p>0.05$), 1% ($p>0.05$) and -1% ($p>0.05$) respectively, during the post-treatment time intervals indicated. Thus, H-7 does not increase corneal endothelial permeability.

TABLE 5A.
Effect of 400 mM Topical H-7 on Corneal Endothelial Transfer Coefficient (k_a)
At 0.5-3.0 Hours

	H-7	Vehicle	H-7/Vehicle
Baseline	4.21 ± 0.15	4.48 ± 0.35	0.96 ± 0.05
Post-Drug k_a	4.19 ± 0.76	4.28 ± 0.55	0.98 ± 0.12
Post-Drug k_a/Baseline	0.98 ± 0.16	0.98 ± 0.14	1.05 ± 0.16

TABLE 5B.
Effect of 400 mM Topical H-7 on Corneal Endothelial Transfer Coefficient (k_a)
At 3.5-6.0 Hours

	H-7	Vehicle	H-7/Vehicle
Baseline	5.13 ± 0.19	5.27 ± 0.29	0.98 ± 0.03
Post-Drug k_a	5.05 ± 0.60	5.18 ± 0.39	0.99 ± 0.12
Post-Drug k_a/Baseline	0.97 ± 0.09	0.98 ± 0.05	1.01 ± 0.13

TABLE 5C.
Effect of 400 mM Topical H-7 on Corneal Endothelial Transfer Coefficient (k_a)
At 0.5-6.0 Hours

	H-7	Vehicle	H-7/Vehicle
Baseline	4.67 ± 0.16	4.80 ± 0.25	0.98 ± 0.02
Post-Drug k_a	4.70 ± 0.60	4.86 ± 0.35	0.96 ± 0.10
Post-Drug k_a/Baseline	0.99 ± 0.10	1.02 ± 0.07	0.99 ± 0.11